A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells.

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Public Summary:
This paper describes an improvement to one of the components in the CRISPR/Cas9 gene editing system. Gene editing in mammalian cells has been revolutionized by the application of bacterial CRISPR/Cas9. The bacterial Streptococcus pyogenes Cas9 protein has been the most highly used CRISPR system. Its use for genome editing requires the presence of the Cas9 protein and a guide that can identify the correct piece of DNA for the Cas9 protein to edit. However, sometimes the guide can lead the Cas9 protein to DNA that is similar to the correct DNA but is not the desired site-this is called "off-target" and can lead to undesirable Cas9 mediated editing events. We have engineered a new Cas9 protein that has far greater specificity for its correct target sequence in the genome, thus dramatically decreasing off-target editing. This engineered Cas9 (HiFi) is widely applicable in both basic and therapeutic applications.

Scientific Abstract:
Translation of the CRISPR-Cas9 system to human therapeutics holds high promise. However, specificity remains a concern especially when modifying stem cell populations. We show that existing rationally engineered Cas9 high-fidelity variants have reduced on-target activity when using the therapeutically relevant ribonucleoprotein (RNP) delivery method. Therefore, we devised an unbiased bacterial screen to isolate variants that retain activity in the RNP format. Introduction of a single point mutation, p.R691A, in Cas9 (high-fidelity (HiFi) Cas9) retained the high on-target activity of Cas9 while reducing off-target editing. HiFi Cas9 induces robust AAV6-mediated gene targeting at five therapeutically relevant loci (HBB, IL2RG, CCR5, HEXB, and TRAC) in human CD34(+) hematopoietic stem and progenitor cells (HSPCs) as well as primary T cells. We also show that HiFi Cas9 mediates high-level correction of the sickle cell disease (SCD)-causing p.E6V mutation in HSPCs derived from patients with SCD. We anticipate that HiFi Cas9 will have wide utility for both basic science and therapeutic genome-editing applications.